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Application No. 09/147,036
Reply to Office Action of November 3, 2004
Amendment Dated March 3, 2005

Amendments to the Claims:

This Listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently amended) A process for presenting a passenger peptide or polypeptide on the surface of Gram-negative host bacteria, comprising
- a) providing a host bacterium transformed with a vector encoding a polynucleotide operatively linked to a promoter, wherein said polynucleotide comprises:
 - (i) a nucleotide sequence encoding a signal peptide,
 - (ii) a nucleotide sequence encoding a passenger peptide or polypeptide,
 - (iii) a nucleotide sequence encoding a protease recognition site,
 - (iv) a nucleotide sequence encoding a transmembrane linker, and
 - (v) a nucleotide sequence encoding a transporter domain of an autotransporter the AIDA protein of *E. coli*, wherein the nucleotide sequence encoding the transporter domain is located downstream from the nucleotide sequence encoding the passenger peptide or polypeptide; and
 - b) cultivating the host bacterium under conditions for inducing expression of the polynucleotide and presentation of the passenger peptide or polypeptide of (ii) on the surface of the host bacterium, wherein the passenger peptide or polypeptide of (ii) is heterologous in

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relation to the transporter domain of (v), and the host bacterium is homologous in relation to the transporter domain of (v).

2-8. Canceled.

9. (Previously presented) The process according to claim 1, wherein the passenger peptide has a length of 4-50 amino acids.

10. (Previously presented) The process according to claim 1, wherein the passenger polypeptide is of eukaryotic origin.

11. (Previously presented) The process according to claim 10, wherein the passenger polypeptide is an antibody or an antigen-binding domain of an antibody.

12. (Previously presented) The process according to claim 10, wherein the passenger polypeptide is the α chain of an MHC class II molecule.

13. (Previously presented) The process according to claim 10, wherein the passenger polypeptide is the β chain of an MHC class II molecule.

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14. (Previously presented) The process according to claim 13, wherein the passenger polypeptide is the β chain of an MHC class II molecule comprising an N terminus to which amino acids for binding are attached.

15. (Currently amended) The process according to claim 1, wherein libraries of variant passenger peptides or polypeptides are expressed in host cells and presented on the host cell-surface, and wherein each host cell expresses one passenger variant.

16-18. (Canceled)

19. (Previously presented) The process according to claim 15, further comprising selecting single passenger peptides or polypeptides from one of said libraries.

20-40. (Canceled).

41. (Currently amended) A process for obtaining a library of bacteria expressing a variant population of surface-exposed passenger peptides or polypeptides, the process comprising:

a) providing at least one vector comprising a chimeric gene obtained by cloning in frame, a nucleotide sequence encoding a signal peptide, a nucleotide sequence encoding a passenger peptide or polypeptide, and a nucleotide sequence encoding a transporter domain for

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an AIDA protein of *E. coli* or a variant thereof having a homology of at least 80% of the AIDA-1 autotransporter domain in at least its β -sheet region, wherein the nucleotide sequence encoding the transporter domain is located downstream from the nucleotide sequence encoding the passenger peptide or polypeptide;

- b) mutagenizing the at least one vector to introduce variation into the nucleotide sequence encoding the passenger peptide or polypeptide;
 - c) transfecting the at least one vector of step (b) into host bacteria capable of stably presenting the passenger peptide or polypeptide on the cell surface;
 - d) expressing the chimeric gene in the host bacteria;
 - e) culturing the host bacteria of step (d) to produce the passenger peptide or polypeptide stably exposed on the cell surface;
 - f) selecting the host bacteria of step (e) with a surface-exposed passenger peptide or polypeptide,
 - g) identifying and characterizing a binding partner for the surface-exposed passenger peptide or polypeptide, and
- wherein the process is repeated several times in order to obtain the library of bacteria expressing the variant population of surface-exposed passenger peptides or polypeptides.

42. (Canceled).

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43. (Previously presented) The process according to claim 41, wherein the passenger peptides or polypeptides have an affinity for a binding partner selected from the group consisting of a ligand, a receptor, an antigen, a toxin-binding protein, a protein with enzymatic activity, a nucleic acid-binding protein, an inhibitor, a protein having chelator properties, an antibody and an antigen-binding domain of an antibody.

44. (Previously presented) The process according to claim 41, wherein the bacteria expressing the surface-exposed passenger peptides or polypeptides have a binding affinity identified by binding to a labeled or unlabeled immobilized binding partner.

45. (Previously presented) The process according to claim 41, comprising introducing a modification into the binding partner of step g) wherein the modification is subsequently detected.

46. (Previously presented) The process according to claim 41, wherein the passenger peptide or polypeptide is chemically or enzymatically modified on the bacterial surface.

47. (Previously presented) The process according to claim 46, wherein the modification is a non-covalent modification.

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48. (Previously presented) The process according to claim 46, wherein the modification is a covalent modification.

49. (Previously presented) The process according to claim 48, wherein the modification is a glycosylation.

50. (Previously presented) The process according to claim 48, wherein the modification is a phosphorylation.

51. (Previously presented) The process according to claim 46, wherein the modification is a proteolysis.

52. (Previously presented) The process according to claim 51, wherein the passenger peptides or polypeptides are selectively released from the bacterial surface by endogenous or exogenous proteases.

53. (Previously presented) The process according to claim 52, wherein the passenger peptides or polypeptides are released by an endogenous protease of the host cell comprising OmpT protease, OmpK protease or protease X.

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54. Canceled.

55. (Currently amended) A recombinant vector encoding a chimeric polynucleotide operatively linked to a promoter, the chimeric polynucleotide comprising:

- a) a nucleotide sequence encoding a signal peptide,
- b) a nucleotide sequence encoding a passenger peptide or polypeptide,
- c) a nucleotide sequence encoding a protease recognition site,
- d) a nucleotide sequence encoding a transmembrane linker, and
- e) a nucleotide sequence encoding a transporter domain for an AIDA protein of *E. coli* or a variant thereof having a homology of at least 80% of the AIDA-1 autotransporter domain in at least its β -sheet region, wherein the nucleotide sequence encoding the transporter domain is located downstream from the nucleotide sequence encoding the passenger peptide or polypeptide;

wherein the nucleotide sequence encoding the passenger peptide or polypeptide of b) is heterologous in relation to the nucleotide sequence encoding the transporter domain of e).

56. (Previously presented) A recombinant Gram-negative host bacterium, wherein the bacterium is transformed with a vector according to claim 55.

57. (Currently amended) A recombinant Gram-negative host bacterium transformed with a

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recombinant vector encoding a chimeric polynucleotide operatively linked to a promoter, the chimeric polynucleotide comprising:

- a) a nucleotide sequence encoding a signal peptide,
- b) a nucleotide sequence encoding a passenger peptide or polypeptide,
- c) a nucleotide sequence encoding a protease recognition site,
- d) a nucleotide sequence encoding a transmembrane linker, and
- e) a nucleotide sequence encoding a transporter domain of ~~an autotransporter~~ the AIDA protein of *E. coli*, wherein the nucleotide sequence encoding the transporter domain is located downstream from the nucleotide sequence encoding the passenger peptide or polypeptide;

wherein the nucleotide sequence encoding the passenger peptide or polypeptide of b) is heterologous in relation to the nucleotide sequence encoding the transporter domain of e), and wherein the host bacterium is homologous in relation to the nucleotide sequence encoding the transporter domain of e).

58. (Previously presented) The host bacterium according to claim 57, wherein the bacterium is an *E. coli* cell.

59. Canceled.

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